Attachment XVI-A

Attachment A: Proposed new chapter of the *Diagnostic Manual for Aquatic Animal Diseases*

REQUIREMENTS FOR SURVEILLANCE FOR INTERNATIONAL RECOGNITION OF FREEDOM FROM INFECTION

A. INTERNATIONAL RECOGNITION OF FREEDOM FROM INFECTION

1. GENERAL PRINCIPLES

General principles are provided below for declaring a country, zone or aquaculture establishment free from infection in relation to the time of last occurrence, and in particular for the recognition of historical freedom.

An essential prerequisite to provide the guarantees required for the recognition of freedom from infection is that the particular Member Country complies with the provisions of Chapter 1.4.3 of the *Code* for the evaluation of the Competent Authorities.

The provisions are based on the following general principles:

- in the absence of infection or vaccination, the animal *population* would become susceptible to clinical disease, or infection, over a period of time;
- the disease agents to which these provisions apply are likely to produce identifiable clinical or pathological signs in susceptible animals;
- an animal *population* may be free from some specified pathogens but not from others
- there are competent and effective personnel of the Competent Authority able to investigate, diagnose and report disease or infection, if present;
- the absence of infection over a long period of time in susceptible *populations* can be substantiated by effective disease investigation and reporting by the Competent Authority of the Member Country.

2. REQUIREMENTS TO DECLARE A COUNTRY, ZONE OR AQUACULTURE ESTABLISHMENT FREE FROM INFECTION WITH A SPECIFIED PATHOGEN

The requirements to declare a country, zone or aquaculture establishment free from infection differ depending on the previous infection status of the country, zone or aquaculture establishment, namely:

- Absence of susceptible species;
- Historically free;
- Last known occurrence within the previous 25 years;
- Previously unknown infection status.

2.1. Absence of susceptible species

Unless otherwise specified in the relevant disease chapter, a country, zone or aquaculture establishment may be recognised as being free from infection without applying *targeted surveillance* if there are no susceptible species (as listed in the relevant chapter of the *Code*, or in the scientific literature) present in that country, zone or aquaculture establishment, provided that the *prescribed biosecurity conditions* have been in place continuously in the country, zone or aquaculture establishment for at least the previous 10 years.

2.2. Historically free

Unless otherwise specified in the relevant disease chapter, a country, zone or aquaculture establishment may be recognised as being free from infection without formally applying *targeted surveillance* when:

- there has never been any observed occurrence of disease;

or

- eradication has been achieved or the disease has ceased to occur for at least 25 years,

provided that the *prescribed biosecurity conditions* have been in place continuously in the country, zone or aquaculture establishment for at least the previous 10 years.

2.3. Last known occurrence within the previous 25 years

For countries or zones that have achieved eradication (or in which the disease has ceased to occur) within the previous 25 years, in addition to the *prescribed biosecurity conditions*, appropriate *targeted surveillance* must have been applied to demonstrate the absence of the infection, consistent with the provisions of Section B of this chapter.

2.4. Previously unknown infection status

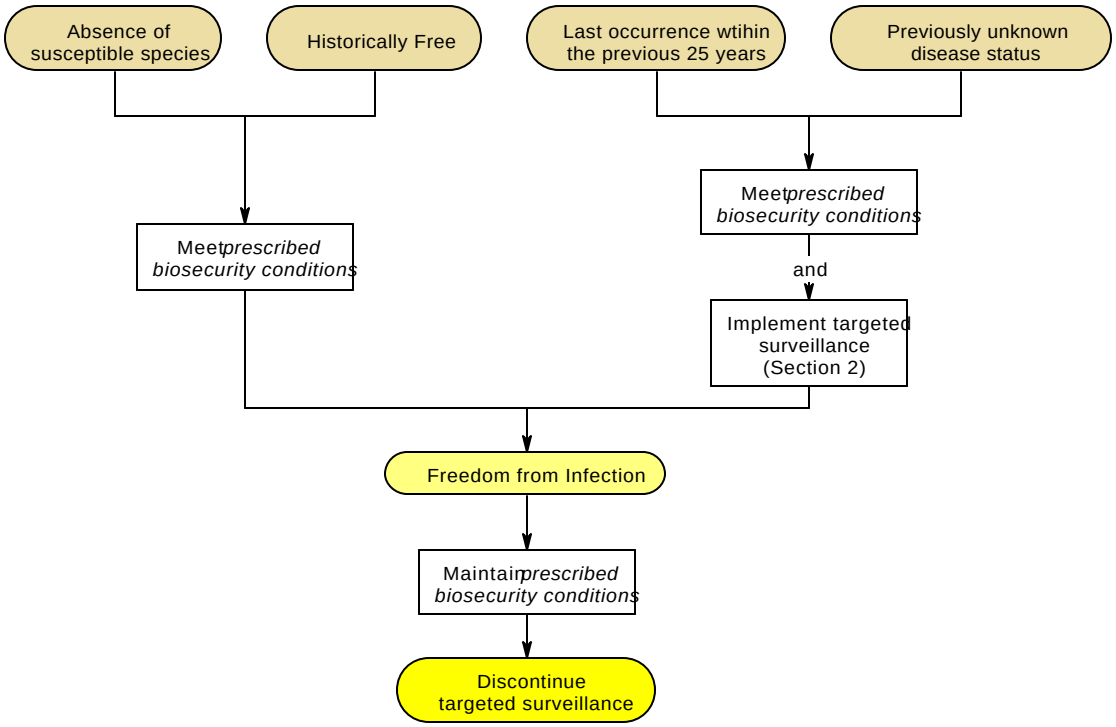
For countries or zones with previously unknown infection status, or which have not previously met the requirements of the Sections A.2.1, A.2.2 or A.2.3 above, the *prescribed biosecurity conditions* must be introduced in addition to *targeted surveillance* consistent with the provisions of Section B of this chapter.

3. GUIDELINES FOR THE MAINTENANCE OF CONTINUED RECOGNITION OF FREEDOM FROM INFECTION

A country, zone or aquaculture establishment that has been recognised free from infection following the provisions of Sections A.2.1 or A.2.2, may maintain its official status as infection-free provided that the *prescribed biosecurity conditions* are continuously maintained.

A country, zone or aquaculture establishment that has been recognised free from infection following the provisions of Sections A.2.3 or A.2.4, may discontinue *targeted surveillance* and maintain its official status as infection-free provided that the *prescribed biosecurity conditions* are continuously maintained.

The different paths to recognition of freedom from infection are summarised in the diagram below.



B. TARGETED SURVEILLANCE FOR DEMONSTRATION OF FREEDOM FROM INFECTION

1. INTRODUCTION

This section provides standards to be applied when demonstrating country, zone or aquaculture establishment freedom from infection, in accordance with the principles of Section A. Standards described in this section may be applied to all *diseases*, their agents and susceptible species as listed in the *Code*, and are designed to assist with the development of surveillance methodologies. More detailed information in each disease chapter (where it exists) of the *Manual* may be used to further refine the general approaches described in this chapter. Where detailed disease/infection-specific information is not available, suitable values should be chosen based on the guidelines in this chapter.

2. GENERAL PRINCIPLES

Demonstrating freedom from infection involves providing sufficient evidence to demonstrate (to an adequate level of *confidence*) that infection with a specified agent is not present in a specified *population*. In practice, it is not possible to prove (i.e. be 100% confident) that a *population* is free from infection (unless every member of the *population* is examined simultaneously with a perfect *test* with *sensitivity* = *specificity* = 100%). Instead, the aim is to provide adequate evidence (to an acceptable level of *confidence*), that infection, if present, is present in less than a specified proportion of the *population*.

Methodologies to demonstrate freedom from infection should be flexible to deal with the complexity of real life situations. No single method is applicable in all cases. Methodologies must be able to accommodate the variety of aquatic animal species, the multiple diseases of relevance, varying production and *surveillance systems*, and types and amounts of data and information available.

The methodology used should be based on the best available information that is in accord with current scientific thinking. The methodology should be well documented and supported with references to the scientific literature and other sources, including expert opinion.

Consistency in methodologies should be encouraged and transparency is essential in order to ensure fairness and rationality, consistency in decision making and ease of understanding by all the interested parties. Applications for recognition of infection-free status should document the uncertainties, the assumptions made, and the effect of these on the final estimate.

3. GENERAL REQUIREMENTS FOR DEMONSTRATION OF FREEDOM FROM INFECTION

3.1. Population

The *target population* to which the demonstration of freedom from infection applies is all individuals of all species susceptible to the infection in a country, zone or aquaculture establishment.

The *study population* may be the same as the *target population* or a subset of it. The *study population* should be (in order of preference):

- The appropriate *study population* as defined in the relevant disease chapter of the *Code* (if such a definition exists),
- A subset of the *target population* that defines a group of animals which, if infection were present, would be most likely to have a higher prevalence of infection than the *target population*. This subset should be defined in terms of:
    - time (e.g. season or month of year);
    - stage of life-cycle or growth period;
    - production system and/or management characteristics;
    - location;
    - readily identifiable physical or behavioural characteristics.
- The same as the *target population*,
- A subset of the *target population* with the same or lower probability of infection. The nature and impact of any biases on the results of the analysis must be considered, documented and taken into account in the analysis.

3.2. Sources of evidence

Evidence of freedom from infection may be based on a number of different sources, including:

- structured, population-based surveys using one or more *tests* for the presence of the agent;
- other *surveillance*, including structured non-random *surveillance*, such as:
    - sentinel sites;

- disease notifications and laboratory investigation records;

- a knowledge of the biology of the agent, including environmental, host *population* distribution, and climatic information;

- history of imports of potentially infected material;

- biosecurity measures in place;

- evaluation of the official services; or

- any other sources that provide contributory evidence that infection is not present in the country, zone or aquaculture establishment.

The sources of evidence used to demonstrate freedom from infection must be fully described. In the case of a structured survey, this must include a description of the sampling strategy used for the selection of *units* for testing. For complex *surveillance systems*, a full description of the system is required including consideration of any biases that may be inherent in the system.

3.3. Statistical methodology

Analysis of data for evidence of freedom from infection involves estimating the probability (a) that the evidence observed (the results of surveillance) could have been produced under the null hypothesis that infection is present in the *population* at a specified prevalence(s) (the design prevalence[s]). The *confidence* in (or, equivalently, the sensitivity of) the *surveillance system* that produced the evidence is equal to 1-a . If the *confidence* level exceeds a pre-set threshold, the evidence is deemed adequate to demonstrate freedom from infection.

The required level of *confidence* in the *surveillance system* (probability that the system would detect infection if infection were present at the specified level) must be greater than or equal to 95%.

The power (probability that the system would report that no infection is present if infection is truly not present) may be set to any value. By convention, this is often set to 80% but may be adjusted according to the country's or zone's requirements.

Different statistical methodologies for the calculation of the probability a , including both quantitative and qualitative approaches, are acceptable as long as they are based on accepted scientific principles.

The methodology used to calculate the *confidence* in the *surveillance system* must be scientifically based and clearly documented, including references to published work describing the methodology.

3.4. Clustering of infection

Infection in a country, zone or aquaculture establishment usually clusters rather than being uniformly distributed through a *population.* Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a zone). Except when dealing with demonstrably homogenous *populations*, approaches to demonstrating freedom must take this clustering into account in the design

and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection.

3.5. Design prevalence

Calculation of the *confidence* of a *surveillance system* is based on the null hypothesis that infection is present in the *population*. The level of infection is specified by the design prevalence. In the simplest case, this is the prevalence of infection in a homogenous *population*. More commonly, in the presence of disease clustering, two design prevalence values are required, for instance, the animal-level prevalence (proportion of fish infected in an infected farm) and the group-level prevalence (proportion of infected farms in the country, zone or aquaculture establishment). Further levels of clustering may be considered, requiring further design prevalence values.

The values for design prevalence used in calculations must be those specified in the relevant disease chapter (if present) of the *Manual*. If not specified for the particular disease, justification for the selection of design prevalence values must be provided, and should be based on the following guidelines:

- At the individual animal level, the design prevalence is based on the biology of the infection in the *population*. It is equal to the minimum expected prevalence of infection in the *study population*, if the infection had become established in that *population*. It is dependent on the dynamics of infection in the *population* and the definition of the *study population*, (which may be defined to maximise the expected prevalence in the presence of infection).

- A suitable design prevalence value at the animal level (e.g. prevalence of infected animals in a cage) may be
  - between 1% and 5% for infections that are transmitted slowly; and
  - over 5% for more contagious infections.

- At higher levels (e.g. cage, pond, farm, village, etc.) the design prevalence usually reflects the prevalence of infection that is practically and reasonably able to be detected by a *surveillance system*. Detection of infection at the lowest limit (a single infected *unit* in the *population*) is rarely feasible in large *populations*. The expected behaviour of the infection may also play a role. Infections that have the ability to spread rapidly between farms may have a higher farm-level design prevalence than slow moving infections.

A suitable design prevalence value for the first level of clustering, (e.g. proportion of infected farms in a zone) may be up to 2%.

3.6. Test characteristics

All surveillance involves performing one or more *tests* for evidence of the presence of current or past infection, ranging from detailed laboratory examinations to farmer observations. The performance level of a *test* at the *population* level is described in terms of its *sensitivity* and *specificity*. Imperfect sensitivity and/or specificity impact on the interpretation of surveillance results and must be taken into account in the analysis of surveillance data.

All calculations must take the performance level (sensitivity and specificity) of any *tests* used into account. The values of sensitivity and specificity used for calculations must be specified,

and the method used to determine or estimate these values must be documented. Where values for sensitivity and/or specificity for a particular *test* are specified in the *Manual*, these values may be used without justification.

Where more than one *test* is used in a *surveillance system* (sometimes called using tests in series or parallel), the overall *test system sensitivity* and *specificity* must be calculated using a scientifically valid method.

Pooled testing involves the pooling of specimens from multiple individuals and performing a single *test* on the pool. Pooled testing is an acceptable approach. Where pooled testing is used, the results of testing must be interpreted using sensitivity and specificity values that have been determined or estimated for that particular pooled testing procedure and for the applicable pool sizes being used. Analysis of the results of pooled testing must be performed using accepted, statistically-based methodologies, which must be fully documented including published references.

3.7. Multiple sources of evidence

Where multiple different data sources providing evidence of freedom from infection exist or are generated, each of these data sources may be analysed according to the provisions of Sections B.3, B.4 (for structured surveys) and B.5 (for complex data sources). The resulting estimates of the *confidence* in each data source may be combined to provide an overall level of *confidence* for the combined data sources.

The methodology used to combine the estimates from multiple data sources:

- must be scientifically valid, and fully documented including references to published material; and
- should, where possible, take into account any lack of statistical independence between different data sources.

Surveillance information gathered from the same country, zone or aquaculture establishment at different times may provide cumulative evidence of freedom from infection. Such evidence gathered over time may be combined into an overall level of *confidence*. For instance, repeated annual surveys may be analysed to provide a cumulative level of *confidence*. However, a single (larger) survey may be able to achieve the same level of *confidence* in just one year.

Analysis of surveillance information gathered intermittently or continuously over time should, where possible, incorporate the time of collection of the information to take the decreased value of older information into account.

4. SPECIFIC REQUIREMENTS FOR STRUCTURED SURVEY DESIGN AND ANALYSIS

One method of generating evidence for freedom from infection is the use of structured, population-based, targeted surveys. In addition to the requirements specified in Section B.3, the following guidelines should be used when implementing and analysing surveys to demonstrate freedom from infection.

4.1. Survey design

The most important *unit* of diagnosis is the *epidemiological unit*. The *population* of *epidemiological units* must be clearly defined.

The design of the survey will depend on the size and structure of the *population* being studied. If the *population* is relatively small and can be considered to be homogenous with regards to risk of infection, a single stage survey can be used.

In larger *populations* where a sampling frame is not available, or when there is a likelihood of clustering of disease, multi-stage sampling is required. In two-stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, farms or villages) are selected. At the second, animals are selected for testing from each of the selected groups.

Stratification may be used in survey design.

4.2. Sampling

The objective of sampling from a *population* is to select a subset of *units* from the *population* that is representative of the *population* with respect to the characteristic of interest (in this case, the presence or absence of infection). Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the *population*, within the practical constraints imposed by different environments and production systems.

Biased or targeted sampling in this context involves sampling from a defined *study population* that has a higher probability of infection than the *target population* of which it is a sub-population. Once the *study population* has been identified, the objective is still to select a representative sample from this sub-population.

4.3. Sampling methods

The survey design may involve sampling at several levels.

For sampling at the level of the *epidemiological units* or higher *units*, a formal *probability sampling* (e.g. simple random sampling) method must be used.

When sampling below the level of the *epidemiological unit* (e.g. individual animal) the sampling method used should provide the best practical chance of generating a sample that is representative of the *population* of the chosen *epidemiological unit*. Collecting a truly representative sample of individual animals (whether from a pond, cage or fishery) is often very difficult.

The sampling method used at all levels must be fully documented and justified.

4.4. Sample size

The number of *units* to be sampled from a *population* should be calculated using a statistically valid technique which takes at least the following factors into account:

- The sensitivity and specificity of the *diagnostic test*, or *test system;*
- The design prevalence (or prevalences where a multi-stage design is used);
- The level of *confidence* that is desired of the survey results.

Additionally, other factors may be considered in sample size calculations, including (but not limited to):

- The size of the *population* (but it is acceptable to assume that the *population* is infinitely large);
- The desired power of the survey;
- Uncertainty or variability in estimates of sensitivity and specificity.

4.5. Data analysis

Analysis of *test* results from a survey shall be in accordance with the provisions of Section B.3 and take at least the following considerations into account:

- The survey design;
- The sensitivity and specificity of the *test*, or *test system;*
- The design prevalence (or prevalences where a multi-stage design is used);
- The results of the survey.

4.6. Quality assurance

Surveys should include a documented quality assurance system, to ensure that field and other procedures conform to the specified survey design. Acceptable systems may be quite simple, as long as they provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the survey design.

5. Specific Requirements for Complex Non-survey Data Sources

Data sources that provide evidence of freedom from infection, but are not based on structured population-based surveys may also be used to demonstrate freedom, either alone or in combination with other data sources. Different methodologies may be used for the analysis of such data sources, but the methodology must comply with the provisions of Section B.3. The approach used should, where possible, also take into account any lack of statistical independence between observations.

Analytical methodologies based on the use of step-wise probability estimates to describe the *surveillance system* may determine the probability of each step either by:

- the analysis of available data, using a scientifically valid methodology; or where no data are available,
- the use of estimates based on expert opinion, gathered and combined using a formal, documented and scientifically valid methodology.

Where there is significant uncertainty and/or variability in estimates used in the analysis, stochastic modelling or other equivalent techniques should be used to assess the impact of this uncertainty and/or variability on the final estimate of *confidence*.